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Papillomas of the External Ear Canal: Report of Ten cases in Chinese Patients with HPV In Situ Hybridization

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Abstract Squamous papilloma is a benign exophytic proliferation which can occur occasionally in the external ear canal. It is widely assumed that the Human Papilloma Virus (HPV) is an etiologic factor of papillomas. Available techniques for detection of HPV genomes include immunohistochemistry, Southern blot hybridization, in situ hybridization (ISH), and polymerase chain reaction. To our knowledge, HPV typing has not been reported on tissue sections of papillomas in the external ear canal. We report HPV ISH analysis in ten cases of papillomas, involving the external ear canal in Chinese patients. These papilloma excrescences were less than 1 cm in diameter, and were benign morphologically. Automated HPV ISH analysis was performed for the hybridization of DNA probes, including both low-risk and high-risk HPV subtypes. HPV ISH results revealed that seven out of ten cases were positive for low-risk HPV (6, 11), three cases demonstrated no hybridization for low-risk HPV probe, and none of the cases revealed any detection of high-risk HPV (16, 18, 31, 33, 35, 39, 51, 52, 56, 58, and 66). On follow-up after 18–29 months (average 24.5 months), eight patients were doing well, with no local recurrence after excision. Two patients were lost to follow-up. Our results confirm that benign papillomas of the external ear canal are associated with low-risk HPV infection with benign behavior and neither recurrence nor high grade dysplasia.

Keywords Squamous papilloma · External ear canal · Low-risk human papilloma virus (6, 11) · In situ hybridization

Introduction

Squamous papillomas are benign neoplasms in the head and neck region, usually affecting the skin, oral cavity mucosa, and upper aerodigestive tract but only a few reports describe involvement of the external auditory canal [1–7]. Though uncommon, this affliction is distributed throughout the population; all ages may be affected with no sex predilection [5].

Papillomas are widely suspected to be associated with the Human Papilloma Virus (HPV). HPV belongs to the family of DNA Papovaviridae, which are small, non-enveloped icosahedral viruses, each with an 8 Kb-long double-stranded circular DNA genome. The double-stranded circular DNA molecule of 7,900 base-pairs is an epitheliotropic virus (a virus that infects epithelial cells) [8]. HPV in situ hybridization (ISH) analysis is performed by using HPV DNA probes to detect HPV DNA in the nuclei of the infected squamous cells.

Certain HPV subtypes have been identified in squamous cell lesions of the head and neck [9–11]. However, their role in the pathogenesis of external ear canal papillomas has not been studied. Such strains of HPV can cause pre-malignant lesions and carcinomas in the areas they infect, and are called “high-risk” types. Others, called “low-risk”

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types, may cause mild cytologic abnormalities and genital warts, as well as laryngeal papillomatosis and oral condyloma accuminata. The most common high-risk subtypes are types 16 and 18, while the most common of the low-risk subtypes are types 6 and 11 [12]. Techniques for detection of HPV in squamous papillomas include immunohistochemistry, Southern blot hybridization, ISH, or PCR. It has been previously reported that HPV was detected in the papillomas of external ear canal by PCR [11]; however, the correlation of HPV infection to micro-anatomic localization and viral distribution of papillomas in this particular site has not been previously described. Our study aimed to identify which group of HPV subtypes is associated with external ear canal papillomas, and also to visualize and localize these virally infected cells.

Materials and Methods

Ten excisional biopsy specimens of the external ear canal (five from the left and five from the right ear canals) were collected from ten Chinese patients (eight male, two female) at the Eye, Ear, Nose and Throat Hospital at Fudan University. Patients' ages ranged from 29 to 70 years (average 53) (Table 1). Squamous papillomas of the oral cavity, ears or cheeks, or facial skin "warts" were not noted in these patients' past history. All tissue was fixed in 10% neutral-buffered formalin and embedded in paraffin. Routine histological examinations with hematoxylin and eosin stain were performed.

Automated HPV in situ hybridization (ISH) analysis was performed on 4 μm thick paraffin embedded tissue sections using a BenchMark XT (Ventana Medical Systems, Tuscon, AZ) for the hybridization of low-risk and high-risk DNA probes. The low risk group DNA probe contains HPV genotypes 6 and 11, while the high risk HPV group contains HPV genotypes 16, 18, 31, 33, 35, 39, 51, 52, 56, 58, and 66. Positive and negative controls were hybridized alongside the study cases. Positive staining was visualized using the precipitating chromogenic reaction NBT/BCIP with a nuclear localization. A positive ISH is defined by a dark blue nuclear stain, in papillary squamous epithelium [10, 13–16].

Results

Papillary excrescences were present in the external ear canal and were usually less than 1 cm in diameter (Fig. 1a) and occasionally ulcerated (Fig. 1b). These papillomas were singular lesions. On histological examination, they consisted of multiple slender, finger-like projections supported by central fibrovascular cores, which were covered by a keratinizing squamous epithelium (Fig. 1c, d). Infected squamous cells exhibit dense dark nuclei with clear cytoplasm, some of which occasionally have bi-nuclei (koilocytic cells). There were mild cellular atypical changes in basal layer of squamous epithelium, but mitoses were infrequently seen (<2 mitoses/10 high power field). No high-grade dysplasia or invasive component was seen. Along the basal squamous epithelium, chronic inflammation was histologically recognized. Inflammatory atypia was also present in several papillomas. The extent of mild cellular atypia and chronic inflammation varied among the cases (Table 1).

Automated HPV ISH results reveal that seven cases were positive for the HPV types 6 and 11 probe (low risk 70%) (Fig. 1e, f), and three cases did not demonstrate hybridization for either high- or low-risk HPV probes. The positive ISH staining pattern ranged from a few dark blue nuclei to diffuse nuclear stain with no cytoplasmic staining.

Eight patients were followed up for 18–29 months (average 24.5 months) with no local recurrence. Two patients were lost to follow-up (Table 1).

Table 1 Summary of ten papillomas of external ear canal in Chinese patients and HPV ISH results

Case No.	Age (year)	Sex	Site	HPV subtypes	Basilar cellular atypia	Koilocytes	Chronic inflammation	Follow up (month)
1	70	M	Left	LR*+	Diffuse, mild	Diffuse	Minimal	Loss of f/u
2	70	M	Left	LR+	Focal, mild	Diffuse	Minimal	29
3	64	M	Right	LR+	Focal, mild	Foci	Minimal	26
4	39	M	Left	LR+	Inflammatory atypia, marked	Scattered	Extensive, ulcerated	25
5	29	M	Right	LR−	Minimal	Rare	Minimal	18
6	67	M	Left	LR+	Focal, mild	Diffusely	Patchy	23
7	48	F	Left	LR−	Mild	Scattered	Patchy	23
8	43	F	Right	LR+	Inflammatory atypia, marked	A few	Extensive	Loss of f/u
9	42	M	Right	LR−	Inflammatory atypia	A few	Minimal	27
10	58	M	Right	LR+	Inflammatory atypia	Scattered	Mild	25

* LR: low-risk group of HPV (6, 11). All papillomas were negative for high-risk HPV, f/u: follow up

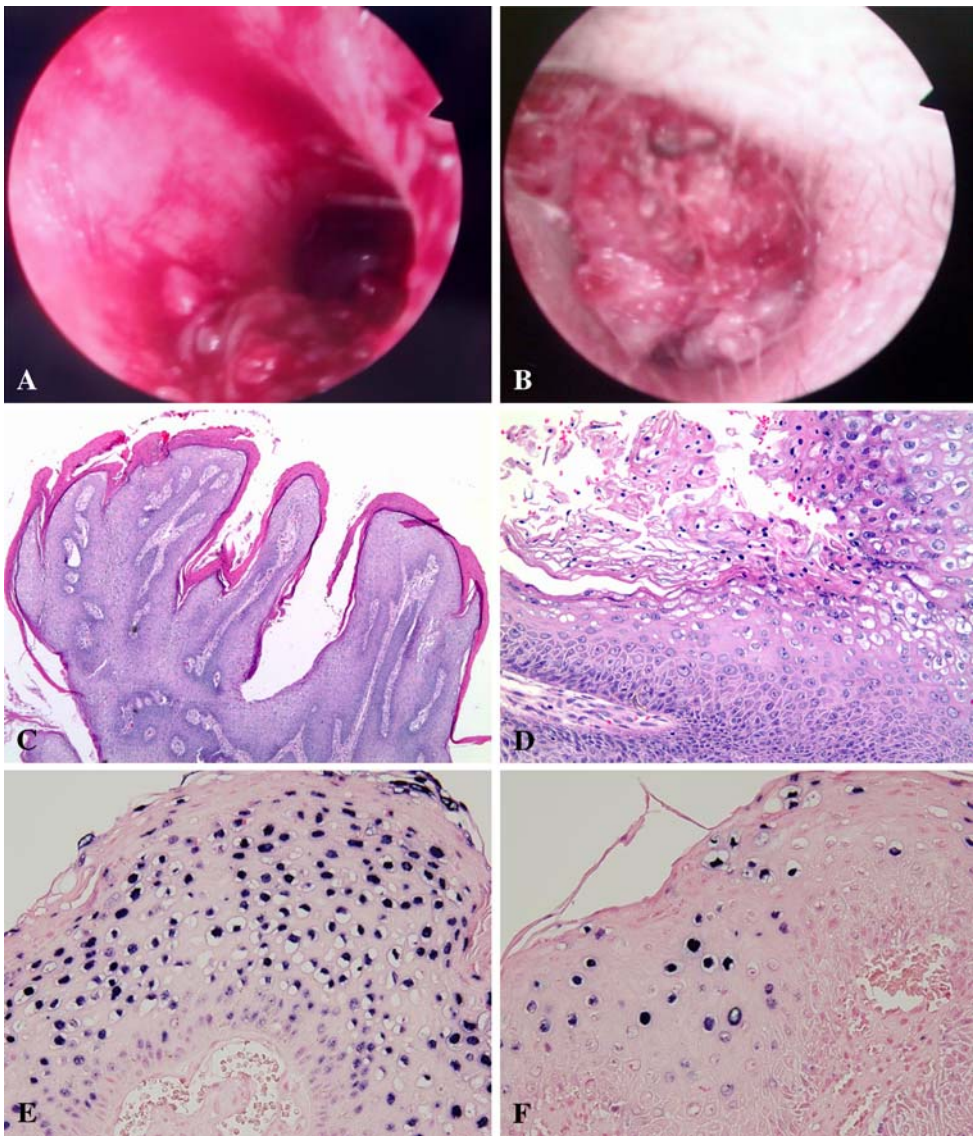

Fig. 1 **a** Photograph from Case 1 shows excrescences of papilloma of the external ear canal, usually solitary. **b** Photograph from the Case 4 shows the papilloma of the ear canal with projections and areas of ulceration. **c** Section of Case 1 shows a squamous papilloma with a central fibrovascular core and covered by keratinizing squamous epithelium. **d** Higher magnification of Case 8 shows HPV infected squamous cells with cytoplasmic clearing and nuclear changes. Basal layers of squamous epithelium show cellular atypia. No significant dysplasia is present. **e** Papilloma of the external ear canal shows numerous squamous cells with viral changes, with positive dense dark nuclei stain for HPV 6/11 low-risk types by ISH (Case 2). **f** Papilloma of the external ear canal shows superficial infected squamous papilloma cells positive for HPV 6/11, low-risk types. Cytoplasmic clearing is present, highlighted in dense dark nuclei (Case 3)

Discussion

Squamous papillomas are frequent in the head and neck region, but they rarely occur in the external ear canal. In an AFIP series extending from 1940 to 1975, the authors found 32 squamous papillomas in the external auditory canal [6, 7]. We report ten cases of Chinese patients with external ear canal papillomas. Our patients presented solitary papillary excrescences in the external ear canal, less than 1 cm in size, with no known association to lesions of the surrounding facial skin (Fig. 1a, b). These papillary growths were indolent and asymptomatic until they were accidentally discovered by the patients. Papillomas of the external ear canal similar to papillomas in other cutaneous sites, can become ulcerated (Fig. 1b), and be inflamed if they are irritated by a local mechanical disturbance such as a scratch.

Histologically, ear canal papillomas form multiple fronds or finger-like projections with a central fibrovascular core covered by stratified squamous epithelium (Fig. 1c). HPV-related infectious cellular changes thought to be related to HPV infection of stem cells within the basal layer of papillomas include cytoplasmic clearing, small dark nuclei, and bi-nucleation with large koilocytes (Fig. 1d). However, koilocytic changes may not be specific when focal only. Our data showed that three cases exhibited a few cells with koilocytic features, but were not detected HPV DNA by HPV ISH (Table 1). How HPV induces cellular proliferation is not well understood [17].

Epidemiologic data have established that sexual transmission of HPV infection plays an important role in the development of genital warts and uterine cervical cancer and is acquired through genital HPV infection with multiple sexual partners [18–21]. Recurrent respiratory papillomatosis in childhood is also considered to be infected through vaginal delivery by infected mothers [8, 22]. From reported series, HPV DNA has been identified in the

airway papillomas, the most common types are low risk HPV 6 and HPV 11, the same types responsible for the majority of genital condylomata or warts. In addition, HPV infection of the oral cavity and pharyngeal area has been thought to be sexually transmitted as well [8, 12, 23]. However, the mode of HPV transmission in the external ear canal is still unknown. It seems unlikely that the external ear canal might easily become directly infected by delivery or sexual contact. It would, however, be possible for a person to become infected by contaminated finger tips or ear picking tools (e.g., Q-tips).

Development of papillomas involves multiple factors, including HPV infection, chronic inflammation and the host immune system [24–26]. Several authors proposed multicentric papillomas in head and neck region. One example is the middle ear papilloma [27–29]. Middle ear papillomas in most cases are thought to be associated with a history of long-term chronic otitis media [29–31]. High risk HPV has been identified in middle ear carcinomas [32]. Welsh et al. [2] reported an external auditory papilloma resulting from dissemination of squamous papilloma by surgical manipulation at the time of surgery for nasal cavity and nasopharyngeal papillomas together with myringotomies. Their report may heighten awareness of papillomas as a potentially transmittable entity to sites outside the respiratory tract, and encourage others to approach these clinical situations with caution. However, none of our patients had had previous surgical procedures.

Since papillomas have specific histologic features, they rarely pose differential diagnostic problems. External ear canal papillomas should be distinguished from other skin lesions including an irritated seborrheic keratosis [33], carcinoma in situ and invasive SCC [34], cholesteatoma [35], skin appendage, and/or other soft tissue neoplasms [36]. However, unlike these lesions, papillomas lack a glandular pattern, and the cytomorphologic characteristics seen in adenomas. Immunohistochemistry for epithelial markers, such as cytokeratin can highlight a papilloma, rather than a neuroma, since the latter is negative for cytokeratin but is positive for S100 protein.

In this study, we applied both low-risk and high-risk groups of HPV DNA probes in paraffin embedded papilloma tissue. After in situ hybridization our data demonstrated that seven out of ten papillomas (70%) were positive for low-risk HPV type 6 and 11 infection. No high-risk type is identified in any cases. Our results are consistent with observations by PCR analysis [11]. In the Xia et al. [11] series, they found papilloma of the external auditory canal (PEAC), which is produced by infection with HPV 6. Unlike skin warts, in which the viral particles are easily demonstrable, the virus particles have not been detected under ultrastructural level. Our HPV ISH analysis was able to visualize the HPV genotypes localized in the infected nuclei. The results clearly show the infected papilloma cells with dense dark nuclei, distributed in infected cells in squamous epithelium (Fig 1e, f). This technique used in our studies is user-friendly, and can be routinely performed on paraffin embedded tissue blocks. Its clinical application can provide diagnostic value; therefore, it can provide a possibility to predict precancerous conditions and help clinical management. For example, in the event that high-risk HPV genotypes are identified and correlate to high-grade dysplasia, the patient needs to be followed up more closely.

Most papillomas can be successfully treated by surgical excision with no recurrence thereafter [3]. The carbon dioxide (CO_2) laser, a new technology, can safely remove surface papillomas in the ear canal. The healing results after laser ablation are quite favorable, with minimal deeper tissue damage or scarring. Most benign squamous papillomas of the external ear have a favorable course with no recurrences, although rare reported cases have undergone an apparent malignant transformation with fatal results [3, 4]. Further research is needed regarding the prevention of transmission of HPV infection. Recently, HPV vaccines have been devised to provide protection against HPV gene products. Some of these have shown promise as immunotherapies for HPV-associated cancers [37–39].

Conclusions

Perhaps, the incidence of external ear canal papillomas is under recognized. These papillomas are associated with low-risk HPV infections that result in a benign clinical course, with no recurrence or malignant transformation. HPV ISH has a diagnostic advantage, which allows us to precisely visualize and localize the HPV genotypes at the infected cellular morphologic level, and therefore can provide critical diagnostic value for better clinical management. Our study is limited to a small number of cases, and does not yet identify any high-grade dysplasia or high-risk HPV genotypes.

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